An Introduction to the Study of Gastrotricha, with a Taxonomic Key to Families and Genera of the Group

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Figure S1. Sampling of marine gastrotrichs of the littoral zone. (A, B) qualitative samples taken by digging several deep holes at the mid-water mark, and transferring the sand with a spoon to a 500 ml plastic jar, (C-F) quantitative samples are taken by inserting corers of known diameter into the sand for 6-10 cm; sand of each corer is then extruded into a plastic jar and fixed on site. Jars are later taken to the laboratory for further processing. Similar techniques may apply to the interstitial forms of freshwater habitats. It is recommended the samples for *in vivo* analysis to be kept at a suitable temperature until processing. (A,B) published with permission.

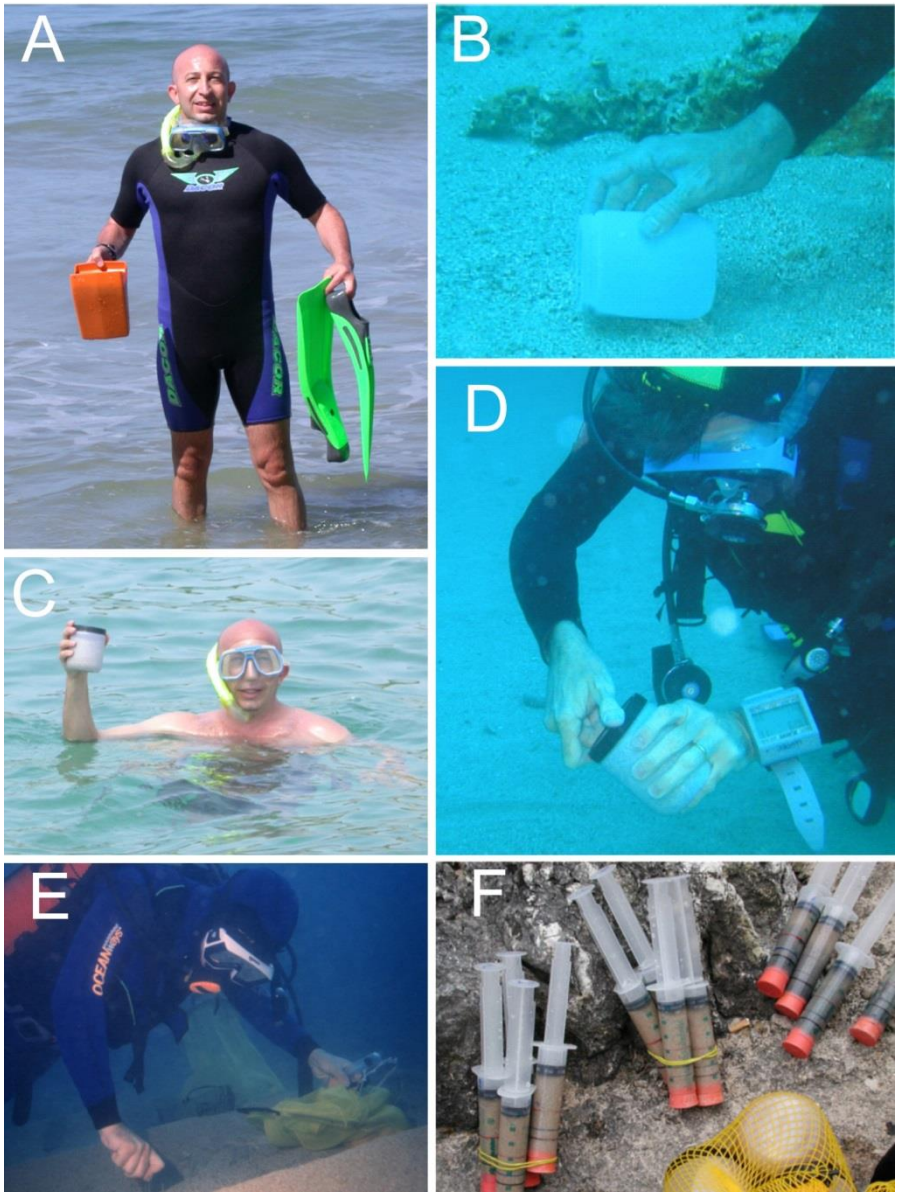

Figure S2. Sampling of marine gastrotrichs of the sublittoral zone. (A-D) qualitative sampling is carried out by skin- or scuba diving; sediments can be collected directly by removing sediment from the top 10-cm layer with a 500-ml plastic jar, which is immediately closed off underwater, (E, F) quantitative samples are taken by inserting corers of known diameter into the sand for 6-10 cm; corers are recovered in shore or on the vessel used for sampling and sand of each corer is then extruded into a plastic jar and fixed on site. (A, C) published with permission.

Figure S3. Sampling freshwater gastrotrichs. (A-F) Benthic, periphytic and semipelagic freshwater species are qualitatively collected by sampling the top layer of the sediment mixed with clumps of vegetation and filtering the water through a 30 µm mesh plankton net. The gastrotrichs enriched samples are then placed in buckets and rapidly transported to the laboratory. (A-F) published with permission.

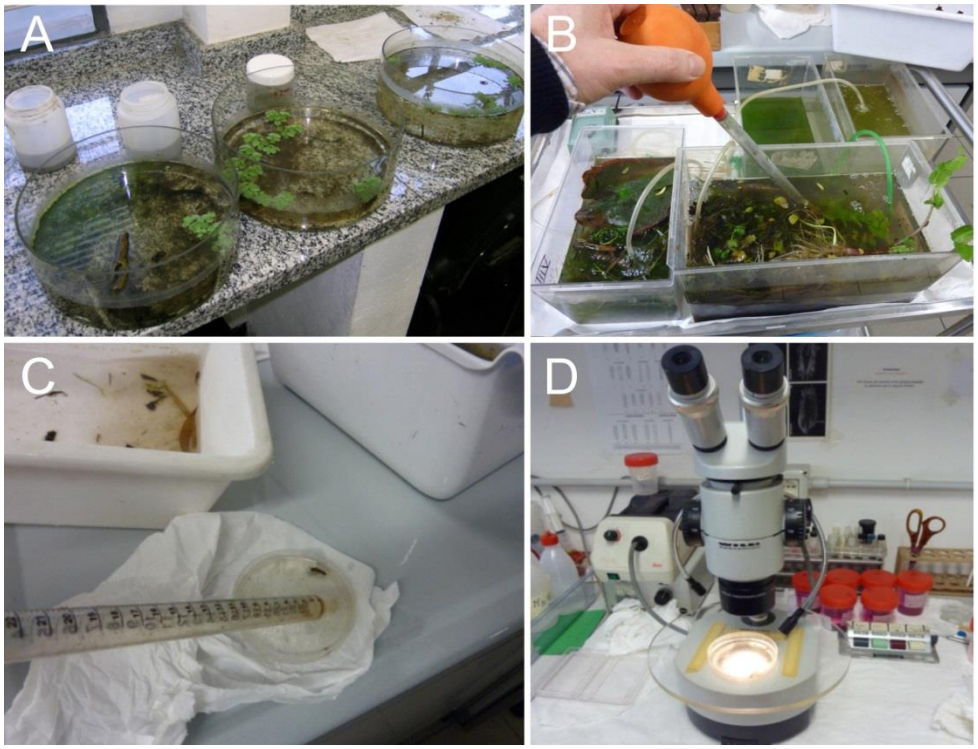

Figure S4. Processing of freshwater sample for *in vivo* studies. (A, B) at the laboratory the freshwater samples enriched with fauna are moved to small aquaria, kept at a suitable temperature and moderately oxygenated with an air stone, (C, D) samples are processed for gastrotrichs by sucking up with a large pipet a small amount of the detritus and the overlaying water and by transferring the sucked material to a large Petri dish (9.5-12 cm); the dish is then scanned for active (motile) gastrotrichs under a dissecting microscope.

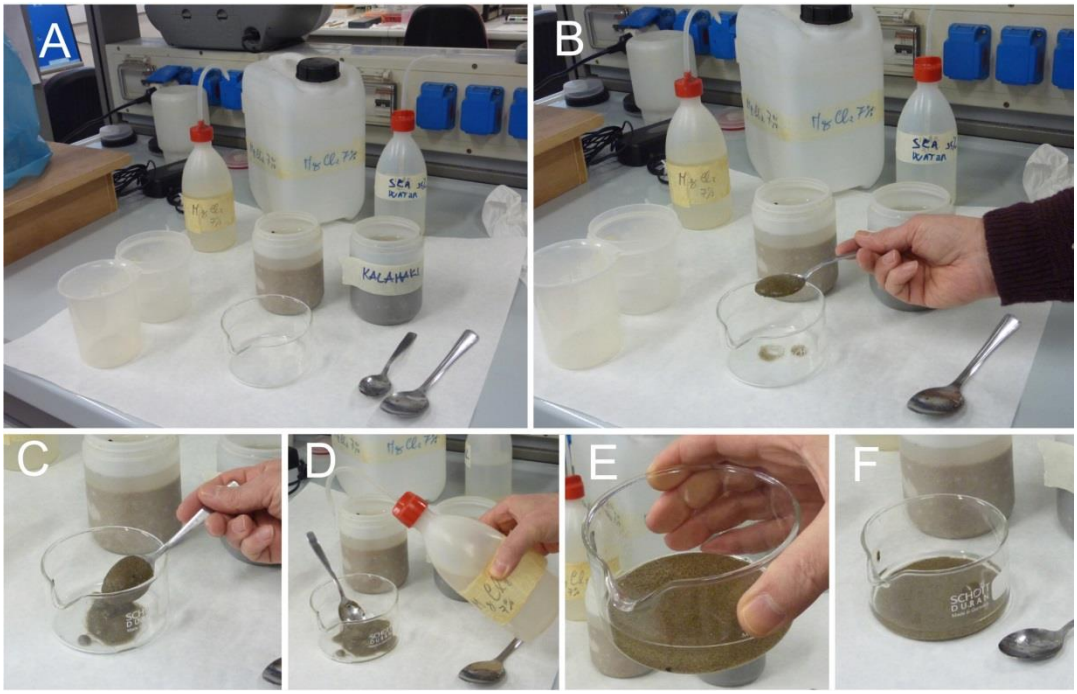

Figure S5. Extraction of interstitial fauna. (A-F) Interstitial gastrotrichs can be extracted (separated) from the sediment by narcotisation with an aqueous solution of $MgCl_2$ (7% marine sample or 1% freshwater sample); to this end, a spoonful of fauna enriched top layer of sand is placed into a small vessel and added with an amount of narcotic solution enough to cover the sand, is then swirled and let to sit for 5 minutes.

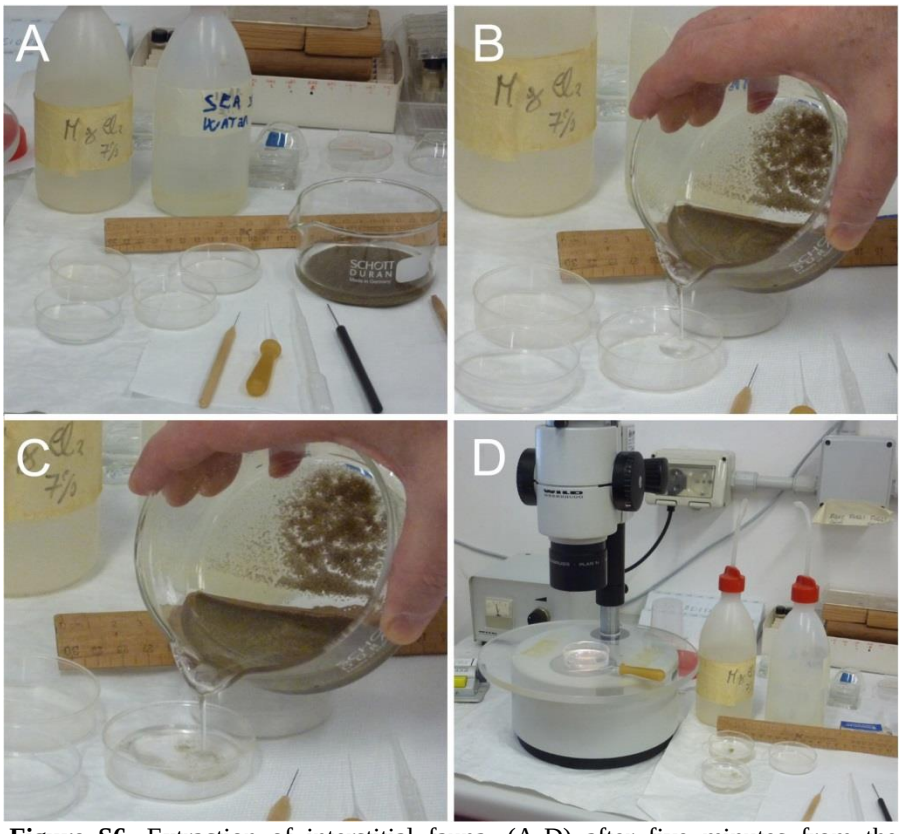

Figure S6. Extraction of interstitial fauna. (A-D) after five minutes from the addition of narcotic, the sample is gently swirled again and the liquid decanted into small Petri dishes (5.5 cm diameter); each petri dish is then scanned for gastrotrichs under a dissecting microscope at 40-50 X magnification, preferably in transmitted light.

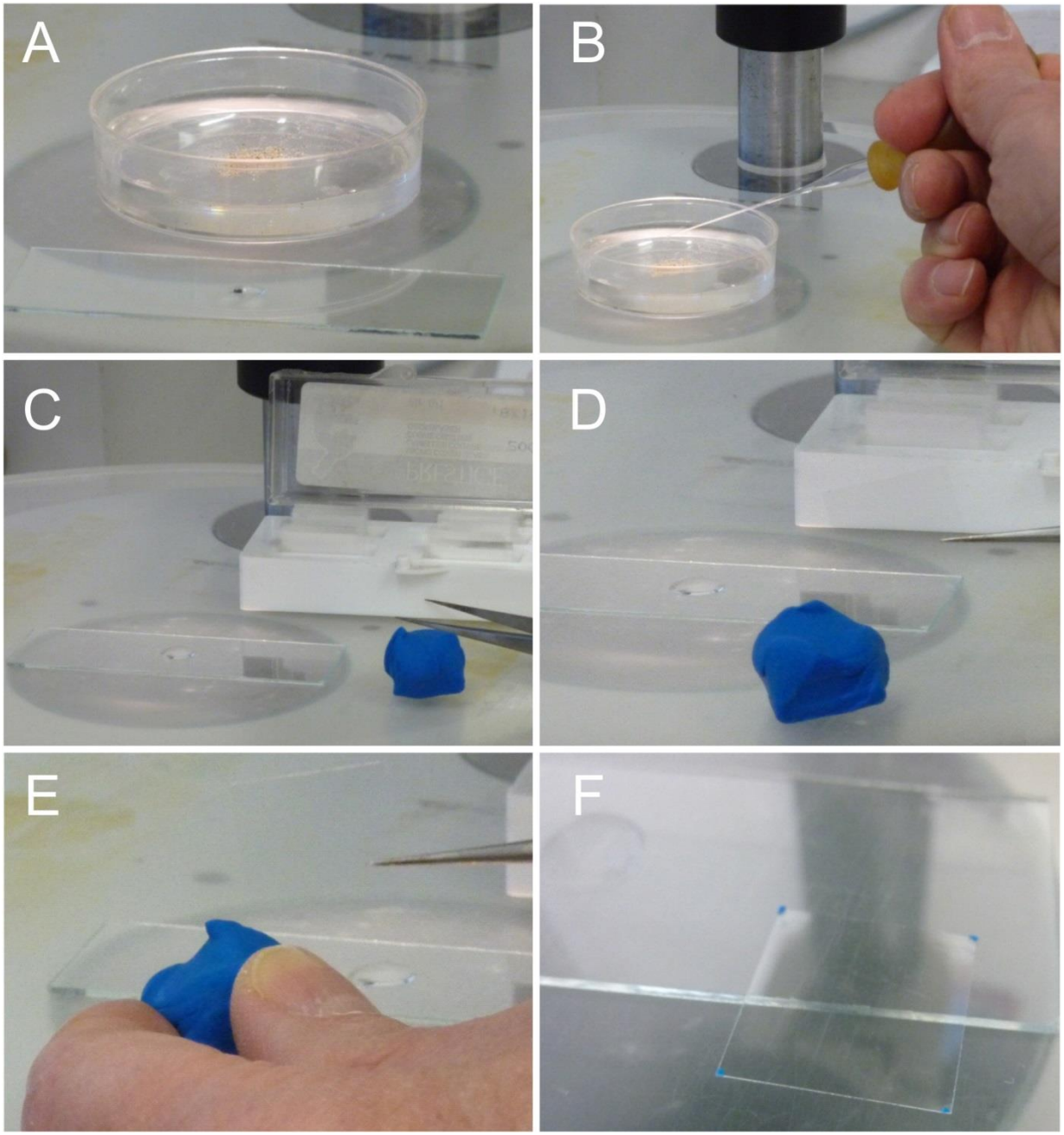

Figure S7. Mounting of the specimens. (A, B) To mount the specimen of interest, a drop of the same medium the specimen is extracted from is put on a microscope slide and a single gastrotrich is transferred to it by using a micropipette, (C-F) a clean coverslip is carefully put on the water; to avoid excessive animal compression, the coverslip should not be used as it is; instead, small modelling clay posts are attached beneath its corners prior it is put in place.

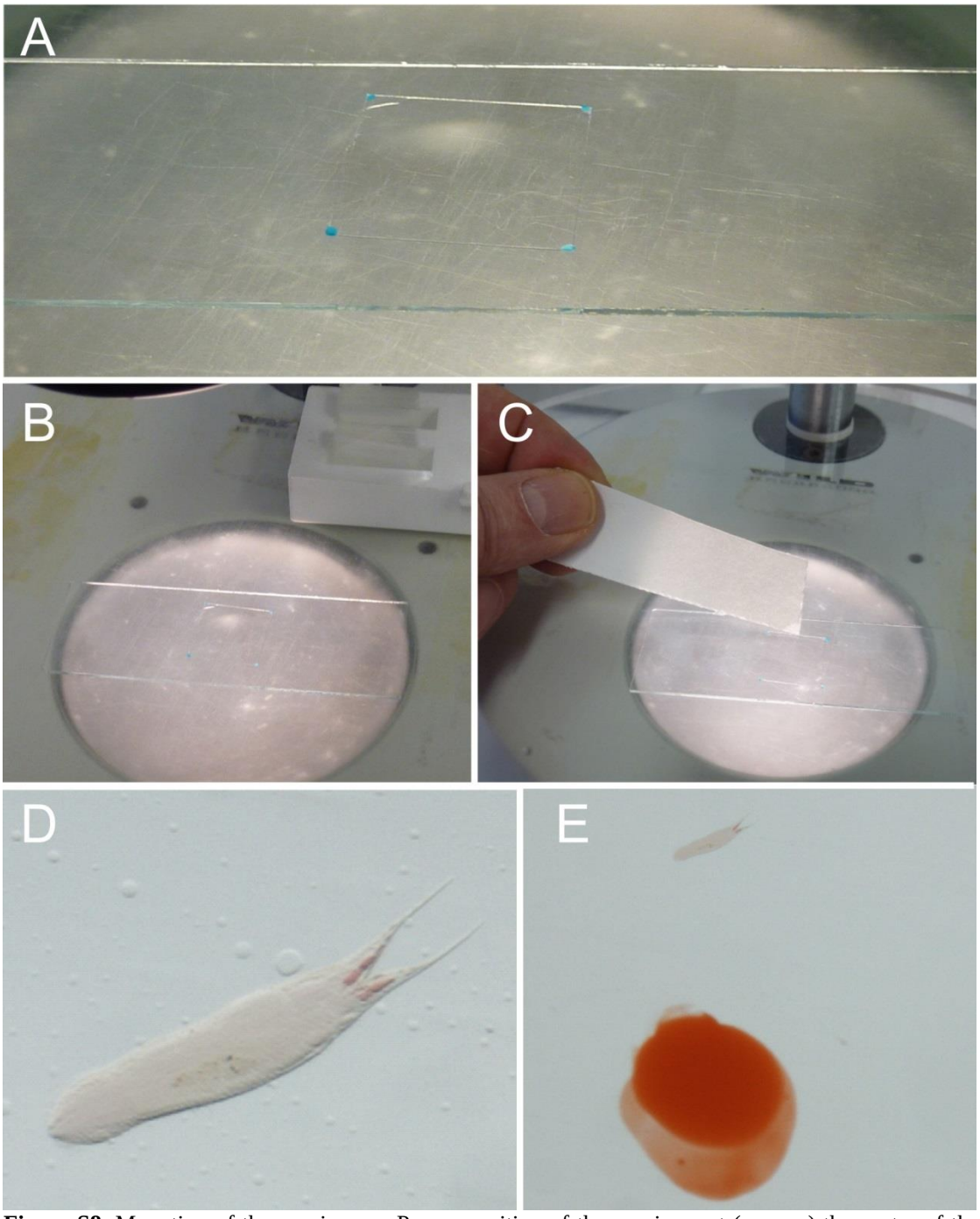

Figure S8. Mounting of the specimens. Proper position of the specimen at (or near) the center of the slide, and a dorso-ventral orientation can be attained by adding a small drop of the liquid medium to the sides of the coverslip or by removing the liquid with a filter paper, a small ink drop near the specimens may facilitate the find of the sample under the compound microscope.

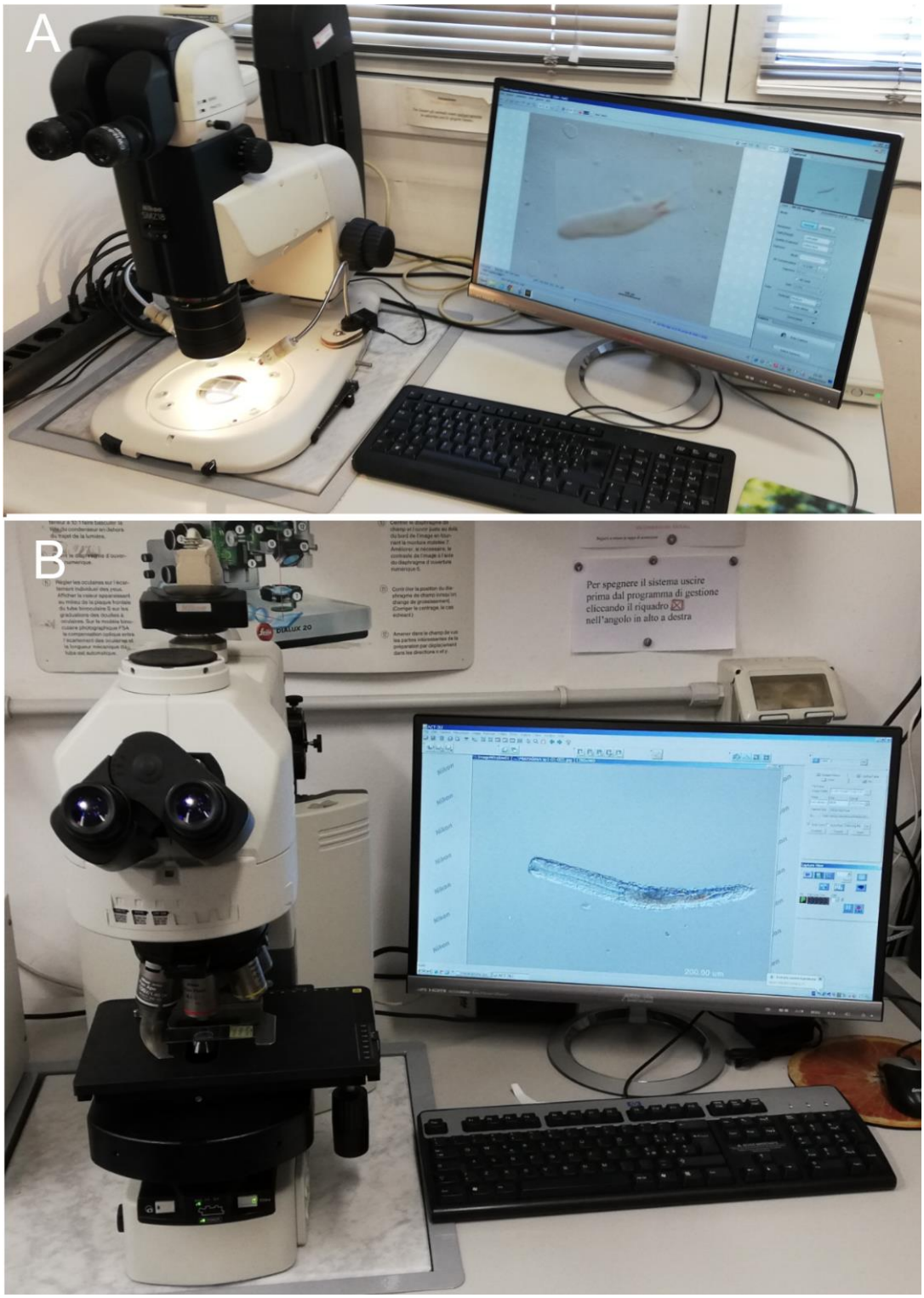

Figure S9. Morphological analysis and documentation. (A) state-of-the-art binocular- and (B) compound microscopes, both are fitted with a high resolution photo-camera. Morphometric data should be acquired on living, relaxed specimens mounted on a microscope slide and covered with square 15-18 mm coverslip. Animals gently compressed between the slide and the coverslip are then observed under a compound microscope, preferably using differential interference contrast optics (DIC). Identification of formalin fixed gastrotrichs can be performed on specimens mounted in water, or better, based on (semi)permanent mounts. However, in many cases permanent mounts, even in the case of uncontracted, well oriented specimens, do not allow a complete taxonomic study, as several diagnostic features deteriorate over time. Consequently, for taxonomic purposes, photo- or high resolution video-sequences of living, relaxed animals may provide better long-lasting documentations of species characters than permanently mounted specimens.

Figure S10. Instructors (background) and students (foreground) participating at the work-shop on marine meiofaunal organisms of Costa Rica with focus on Gastrotricha (and Kinorhyncha), held in January 2019 at the CIMAR (University of Costa Rica). Published with permission.